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Comparing synthetic and natural grasslands for agricultural production and ecosystem service

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Abstract
Whilst the concept of ecosystem service is relatively new, the importance and benefits of natural grasslands to the environment has been long established. These complex bio-diverse ecosystems, as well as sustaining rich communities of flora and fauna, provide a range of environmental benefits including water, nutrient, and carbon capture. However, the perpetuity of natural grasslands and their associated benefits are under increased threat from pressures to feed and house our increasing population, urban expansion, and also through climate change. Regular ploughing and re-sowing of grasslands has led to soil erosion, depletion of scarce nutrient resources, pollution of our waterways, and releases of harmful greenhouse gases, the latter in particular exacerbated by livestock agriculture. A response is necessary both to reduce negativee impacts of agriculture on the environment and wherever possible to engineer a positive ecosystem service. The genomic and phenomic diversity available in grass and clover species and further access to novel variants through hybridisation with wild-type relatives with suitable technologies to assist in their selection, provide alternatives to current plant varieties and increased capacity for efficient and 'climate-smart' agricultural practice. Holistic approaches to plant breeding can produce varieties that both safeguard agricultural production and provide some valuable ecosystem service.

Introduction: Among diverse grassland ecosystems, it is necessary to distinguish climatically determined grasslands, where water availability is insufficient for development of forest ecosystems and where natural vegetation remains in dynamic equilibrium with herbivores (Lauenroth, 1979), from the anthropogenically-generated grasslands, located mainly within temperate climate regions, where woody vegetation is excluded and herbaceous plant communities maintained by appropriate human intervention and by livestock agriculture. It is possible to further divide the latter grassland type into long-term naturalized grasslands and those that are cultivated, which differ according to level of intensification. However, all grasslands are multifunctional and to different degrees are capable of playing important roles in agronomic, economic and social activities. Whilst an increase in grassland production in terms of its provision as safe, healthy and economically sustainable fodder for livestock consumption, was viewed as an important priority for national security, and still remains a priority, it is also important to acknowledge the important role of grassland ecosystems in all our lives, from the air we breathe to the water we drink, and wherever possible, manage grassland ecosystems to maximise their potential to deliver environmental benefits. The concept of "ecosystem services" was outlined within the Millenium Ecosystem Assessment (2005) (http://www.maweb.org/en/index.aspx). A recent review of 17 grassland biodiversity

experiments revealed that 84% of 147 grassland species displayed ecosystem functions, as measured, at least once (Isbell *et al*., 2011), and while species may appear redundant under one circumstance, they may otherwise function fully in another, leading to the use of species mixtures to help ensure the expression of multiple ecosystem services, particularly when challenged by changing environmental conditions. Similarly for grassland agricultural production, it is normal practice both in the UK and in Ireland to re-sow grasslands with variety mixtures to encourage resilience to predictable stresses, long growing seasons and ultimately sustainable yields.

Whilst the 'market value' of grasslands to agriculture is obvious through the production of meat, wool and milk from grazing animals, they are being increasingly recognised at the global scale for their non-market contribution to carbon sequestration, prevention of soil erosion and for genetic conservation. Other more subtle ecosystem-supporting services include pollination processes, improved soil structure, decreased nutrient leaching and nitrogen fixation. Ecosystem services may be produced *in situ* e.g. through biomass production and carbon sequestration, or adjacent to other habitats and organisms e.g. preventing leaching of nutrients downhill into rivers, streams or wetlands, or a source of flowers for pollinators from other locations.
Many grasslands are 'highly improved' and as a consequence have significantly reduced ecosystem services compared to semi-natural or natural grassland ecosystems. The plight of semi-natural and natural grasslands is well known with a loss of at least 98% of our wild flower meadows in Britain over the last 50 years (Peterken 2013). Their fragmentation and degradation have reduced their potential for ecosystem service significantly.
For grasslands as with forest ecosystems, plant-soil interactions involve different and complex cycling elements, particularly of carbon (C), nitrogen (N), and phosphorus (P). These operate (i) in plants, where N, P and C are combined in organic matter synthesis, accumulation and long-term sequestration in soils; and (ii) in soils, where microbes feed abundantly on C and recapture and recycle mineral N and P. In most circumstances, grasslands can sequester C, N and P for relatively long-term periods, so contributing to the atmospheric CO_2 sink, and reducing release of N compounds into water and atmosphere and their associated environmental risks. Grasslands are land-use systems that can be very favourable for environmental preservation. However, this idealistic view has to be tempered as livestock decouple C and N–P cycles, through their urine and faeces depositions and their methane (CH_4) emissions, offsetting, sometimes to a significant extent, the beneficial effect of grassland vegetation–soil interactions.

Alternative viewpoints persist between ecologists and crop geneticists as to what extent it is possible to reduce the detrimental impacts of grassland agriculture on the environment by combined use of best farming practice and the inclusion of new and more sustainable grassland crop varieties. Entrenched viewpoints are sustained by some crop geneticists' naïve understanding of ecosystem complexity and certain field ecologists' ignorance of the potential of new plant breeding technologies, as well as by others whose priorities, for whatever reason, may differ significantly. Whilst risking being considered naïve, an argument is presented herein where a 'win-win' scenario might be achieved, but, as authors with different viewpoints ourselves, we counter-balance the argument by specific reference to the greater service diversity obtained from semi-natural grasslands, both by their species diversity and the far more stable soil system they support.

Through the incorporation of new and appropriate technologies and germplasm, it is proposed on the one hand that there are significant opportunities available to 'untap' potential

for ecosystem service from novel grasslands, whilst also providing productive crops considered fit for agricultural purpose. However, before these are outlined it should be clarified that the authors consider any new option for grassland agriculture that might safeguard the needs of both agriculture and the environment, will not in any way displace current priorities to safeguard existing semi-natural grassland ecosystems, whose complex and diverse benefits are not likely to be easily reproduced.

Semi-natural grasslands and their ecosystem services: The value of semi-natural grasslands for ecosystem service should never be underestimated. To paraphrase the British Ecological Society Bulletin Bogbean Bennet Cartoon-strip (http://homepages.abdn.ac.uk/g.j.pierce/pages/bbb.htm), any attempt to compensate for the destruction of a habitat by reproducing something similar elsewhere is like "moving the Mona Lisa" by scraping away all the paint on the canvas and reconstructing it elsewhere: the paint may be the same, but the interconnectivity of the paint flakes is totally lost. Ecosystems are highly complex. For the most part we study only certain aspects such as carbon sequestration, productivity, or species composition; be it birds, mammals, invertebrates or plants. The soil may be studied for respiration (productivity), arthropods, or fungi, but the full ecosystem *in toto* is virtually impossible to describe and quantify. So the ability to recreate it is not, in reality, a feasible option.
In Ireland, as in Britain, grasslands are a derived habitat, maintained by farming practice, either by grazing or cutting. The last 50 years or more have seen widespread intensification, largely driven by EU policies and incentives (Hickie *et al.,* 1999). Grasslands were traditionally managed by low-intensity farming and semi-natural grasslands differed in species composition as a consequence of e.g. soil pH or water content (O'Sullivan, 1982). The EU drive for productivity beginning in the 1970s led to the ploughing up of grasslands for rotation crops and re-seeding for silage, with a concomitant reliance on chemicals, including fertilizer application. These changes resulted in the reduction of semi-natural grassland and therefore plant species diversity in a grassland landscape. In particular, the traditional extensive practice of cutting meadows for hay has declined substantially in recent decades (Peterken, 2013). Semi-natural grasslands have thus imperceptibly become very rare in Ireland, Britain and mainland Europe (Fuller, 1987, Baldock, 1989, Feehan, 2003, Sullivan *et al*. 2010). Their conservation is now considered a priority and forms part of EU-, as well as national policy; the EU Habitats Directive lists 31 semi-natural grassland habitats in Europe, and national programmes are focused on conserving the range of these habitats that still remain (Critchley *et al.,* 2003, EEA, 2004, Stevens *et al.* 2010; O'Neill *et al*., 2013).
Aside from the ethics of conserving increasingly rare species-rich habitats, it is now seen as important to quantify their contribution to the environment as ecosystem services. This relatively recent concept relates to the current need to put an economic value on our environment, since, if it affects us economically, it may induce governments to take action. The drive to be productive and increase profit margins is influenced in this way, but the economic losses resulting from agricultural intensification also require evaluation (Purvis *et al.,* 2008, Dreschler *et al.,* 2010).

Individual plant traits in grassland studies have come to the fore in relation to ecosystem services from semi-natural and natural grasslands in Europe. A Europe-wide study named VESTA (Garnier, *et al*., 2007) involving 11 European sites illustrates that experiments that can isolate direct effects of climate and land use from indirect effects, such as changes in community functional composition, advance our understanding of the role of plant traits as linkages between environmental change and ecosystem properties. Inclusion of environmental variables such as climate, soil, and disturbance in quantitative analyses makes

it possible to test hypotheses about the pathways that determine ecosystem properties through modification of plant traits in communities.

Value and productivity of species-rich swards; Arguments for intensive highly-productive agriculture often overlook the fact that grasslands rich in biodiversity have been shown to be more productive than species-poor swards (Tilman *et al.,* 1996; 1997), especially grasslands with high forb diversity and resultant complementarities of resource use (Hooper *et al.* 2005; Bullock *et al*. 2007). The number of legumes alone does not account for a yield increase, instead the wide range of temporal and spatial growth patterns exhibited by different forbs maximises the use of resources and can increase fodder value (Hofmann and Isselstein, 2005). Forb diversity is a key factor in the provision of nutritious hay, particularly for horses –that do not eat silage (Allison and Day, 1999). The high quality of the Burren species-rich grasslands is widely known and farmers from the Irish midlands move animals onto the High Burren to increase bone and muscle quality before fattening on richer soils in the spring (Dunford 2002, Williams *et al.* 2009).
The experimental addition of species number on prairie grassland experiencing a fluctuating climate created greater below-ground biomass and temporal stability that in turn, enhanced ecosystem services such as fodder provision and biofuel production (Tilman *et al.*, 2006a). Over ten years, the experimental manipulation of native grassland perennial mixtures (Low Input High Diversity Grasslands, LIHD) of varied species number, demonstrated that these required much less pesticide and fertilizer applications than conventional monoculture crops such as corn and soybean, so were less costly to produce in terms of fossil energy inputs (Tilman *et al.* 2006b). Carbon sequestration in the grassland soils was very high, whereas that of the annual crops was negligible, and would remain so over time. The more diverse the grasslands, the more biomass they produced. As a result, high-diversity grasslands had increasingly higher bioenergy yields that were 238% greater than monoculture yields after a decade (Tilman *et al.* 2006b). The LIHDs were also more beneficial in that they could be grown on degraded soils where no monocultures could easily be grown.
Using an agro-ecosystem approach, the full benefits of a balance between crops, pests and their predators can be evaluated (Altieri, 2008), in contrast to the relative efficiency of a more industrialised approach to agriculture, where the real costs of fuels and chemical application need to be factored in, as well as the relative pest vulnerability of monotypic crops, including intensively-farmed grasslands. The advantages to the environment of organic farming must not be discounted (Niggli et al., 2007), especially as organic farming yields are often no less than those of non-organic farming, contrary to widely-held belief (Badgley et al., 2007). On the other hand, in relation to ecosystem services, it is important to address the range of these that biodiversity can offer, including habitat, as well as species (flora and fauna) diversity (Council of the European Union, 2010).

Pollinator services. Forbs in a grassland sward are important, as these are predominantly insect-pollinated (as opposed to wind-pollinated grasses) and therefore provide both pollen and nectar, for a range of dipteran and other invertebrates (Williams 1988; Branquart and Hemptinne 2000, Peterken 2013). These in turn provide essential pollination for crops, notably oilseed rape, but also fruit, in particular nectar-producing trees such as apple and pear (Free 1993). The recent decline in bees, both the honey bee due to diseases, and bumble and solitary bees, has caused much concern (Williams 1982, Williams *et al*. 1991, Carvell 2002). This has been attributed in large part to loss and fragmentation of semi-natural habitat with services not available in arable fields (Carvell et al 2006). The reduction of a sustained source of food for these species is likely to be a factor in their decline, since once early-flowering

crops such as oilseed rape finish flowering, there must be a series of other species to provide food until the late autumn (Prŷs-Jones and Corbet 1991; Carvell 2002). The type of plant species also affects the pollinator species, since early-flowering annuals provide less nectar and may be more suitable to syrphids and butterflies, but bumblebees require a greater nectar supply and favour perennial forbs (Fussell and Corbet 1991a). Thus re-seeding arable or re-sown field margins may not benefit bumblebees, despite providing nectar-producing flowers (Fussell and Corbet 1991a). Semi-natural grasslands not only benefit nectar- and pollen-feeeders, but enable ground-nesting species such as bumblebees to establish colonies (Fussell and Corbet 1991b). Ant-hill building species such as *Lasius flavus* also only survive in un-ploughed old pastures for the same reason (King 1977).

Remote sensing to measure Ecosystem Services: It is becoming customary and deemed essential to employ new high-throughput technologies to enable the monitoring of large land areas for ecosystem service. Habitats are mapped for their potential for ecosystem services in accordance with definitions that arose out of the Millennium Ecosystem Assessment in 2005 (http://www.maweb.org/en/index.aspx). These are classified as either supporting (primary production, nutrient cycling and soil formation), provisioning (food, fuel, water), regulating (climate, flooding, disease), or cultural (aesthetic, spiritual, educational). Grassland habitats contribute value to all of these, although all too often, the cultural contribution is overlooked. In a study of the Spanish coast, the aesthetic contribution that was shared by a range of habitats was evaluated at >25% (Brenner *et al*., 2010), as proof of its value.
The use of satellite images to identify habitats has been in use since the advent of the LANDSAT satellite series launched in 1982 (Xie *et al*., 2008). As more satellites became available and resolution improved, extensive studies have been undertaken on large territories for land-use mapping e.g. the EU Corine Land Cover initiative (http://www.eea.europa.eu/publications/COR0-landcover). In the UK, there is a bespoke land-cover/land-use mapping initiative using Landsat imagery by Centre for Ecology and Hydrology (CEH). This has identified several improved, semi-natural and natural grassland categories at the national scale. This is updated regularly, most recently in 2007 (http://www.ceh.ac.uk/landcovermap2007.html). However, for UK grasslands, the overall accuracy of the mapping can be very low, as little as 34% for some categories, due to the changing temporal nature of natural and semi-natural grasslands, and the impact of management practices such as grazing, silage removal and fertiliser application.
The advantage of using remote sensing to assess habitat quantity and quality is that it is repeatable over short time scales and covers large areas. China has been estimating the value of grassland ecosystem services country-wide using remote sensing (Jiang, 2007).,using non-market services such as measures for O_2 released, CO_2 fixed, control of soil erosion, water storage, nutrient recycling, reduction of pollution, and Net Primary Productivity. Results showed that habitats such as shrub-meadow complexes and upland grasslands provided the largest ecosystem service, while desert steppe and alpine desert provided the lowest.
Mapping at the landscape scale to define indicative habitat type and area can help with conceptual models of ecosystem services such as pollination. Classification of suitable satellite imagery can define grasslands of all types in terms of area and quality within a range of certainties. From these sources, patch and population-level attributes such as floral density, patch size and patch isolation can be determined, and will influence the interactions of the target plant communities with pollinators. A recent publication has promoted the use of a model called the Mobile-Agent-Based Ecosystem Service (MABES), where ecosystem services are reliant on a mobile organism to deliver a particular service (Kremen *et al.*, 2007). One worked example is pollination, but the model is being adapted for other tasks such as pest control and seed dispersion. Pollination is becoming an increasingly important

ecosystem function due to the decline in bee populations worldwide. From a landscape perspective, a recent meta-analysis found a significantly negative effect of habitat fragmentation on pollination of plants, and a strong correlation of this effect with reproductive success (Aguilar *et al*., 2006).
Thus the fragmentation, and more specifically the isolation, of species-rich grasslands has wider negative effects on the ecosystem services they provide. On a landscape scale, agricultural intensification results in increased size of arable fields, decreased crop and weed diversity, and the loss and fragmentation of valuable natural to semi-natural perennial habitats such as agroforestry, grasslands and old fields. However, with more holistic grassland management at an integrated landscape-scale some more positive effects of agriculture on pollinator communities may occur. For example, in regions where the presence of low-intensity agriculture increases rather than decreases habitat heterogeneity within the foraging range of bees (e.g. <2 km), such as farmed landscapes that include relatively small field sizes, mixed crop types within or between fields, and patches of non-crop vegetation, such as hedgerows, fallow fields, meadows, and semi-natural wood or shrub-lands, it may be beneficial for biodiversity and ecosystem services (Bignal and McCracken 1996; Sullivan *et al.* 2011). Increased ecosystem services by grasslands needs to be properly managed and evaluated (Kremen *et al*., 2007). For instance, what is required to best configure natural habitats within agricultural landscapes to promote population persistence of bees? How do the economic costs of these management practices compare to the benefits from enhanced pollination?

Breeding Grasslands for Crop Production and Carbon Sequestration: A major aspect of ecosystem service by grassland is their role in C sequestration and in mitigating rise in atmospheric CO_2 levels. Whether or not an ecosystem accumulates or loses carbon (both above and below ground) is a function of inputs and outputs. Sequestered carbon can be defined as the difference between gross primary productivity and ecosystem respiration, which in turn is the sum of plant respiration and heterotrophic respiration of non-photosynthetic organisms. This has been termed net ecosystem productivity (NEP) (Chapin *et al.*, 2006). The final rate of accumulation or loss of carbon in a particular ecosystem, in addition to NEP, will depend on external deposition of C (such as inputs of organic manures and dissolved C in rain water) and also losses through erosion, removal (harvesting) and non-biological oxidation through fire or UV radiation (Lovett *et al.*, 2006).
Grass crop breeding that accomplishes increased growth and turnover, both above and below ground provides enhanced opportunity for C deposition. The original source of soil organic matter (SOM) is plant biomass, both above-ground, which decays in the litter layer and can be incorporated into the soil profile, and below-ground through turnover of the root system and as root exudates (Bardgett *et al.*, 2005). Various plant traits can affect the quantity and the quality e.g. C:N ratio of SOM, which in turn can affect its decomposition rates and the level of carbon retained as soil organic matter (De Deyn *et al.*, 2008). Traits that increase the rate of growth of above ground biomass, such as increased rates of photosynthesis, are often associated with a shorter lifespan and high nutrient demands, but this is not always the case. Often associated with high rates of photosynthesis and biomass production is a higher quality of the litter which may lead to a faster turnover rate (Aerts and Chapin, 2000). For perennial crops such as grasses, their strategies for persistence and survival over years requires entry into cycles of growth in spring and early summer followed by reduced foliar and rooting growth in autumn. This may include periods of total growth cessation in preparation for winter survival. These annual growth cycles and associated growth conditions will inevitably impact on the rates of plant C deposition into soils. New strategies in Irish grassland agriculture that aim to make use of rising winter temperatures encourage whole-year growth

and production. However, these have risk as severe crop damage may result from any onset of a harsh winter, and in any case will likely affect rates of accumulation of SOC. There is often trade-off between the amounts of biomass produced and its decomposition, characteristics which may affect soil organic pools. Slower growing plants, in nutrient-poor environments, contribute to soil organic matter more on the basis of the recalcitrance of their organic matter with high C:N ratios leading to slower rates of decomposition. In ecosystems with sufficient light and nutrients, and with high plant growth rate, the proportion of non-harvested biomass, and management regime such as cutting or grazing (where C is returned to the soil via dung) are major factors influencing levels of soil carbon. Grazing (defoliation) intensity and nutrient supply affect the balance of the major C fluxes and are best characterized in terms of the mean leaf area sustained over the growing season. 'Losses' of matter in respiration (approximately 40–50% of 'gross' photosynthesis) increase with the gross C uptake, of which 25% is associated with the energy and mass inefficiency of synthesis of new tissues, and the remaining 25% with the 'maintenance' of existing biomass (Thornley and Johnson, 1990). In perennial ryegrass, the longevity of leaves is approximately 30 days at 15^0C (Parsons *et al*., 2011).
Crops have been developed with different traits and altered biochemistry from the wild type, through selective breeding programmes, hence leading to different litter qualities. Grasses with higher water soluble carbohydrate (WSC) contents, known as high sugar grasses (HSG), have been the focus of breeding programmes at IBERS (formerly IGER) to improve grassland productivity for meat and milk production (Humphreys and Theodorou, 2001). The impact of HSG and their potential for C sequestration is being investigated in collaboration with Rothamsted Research but currently the "jury is out". The increased content of simple carbohydrates of HSG, as opposed to more recalcitrant compounds such as lignin, presents less of a challenge to decomposer organisms, a trait that also makes them more palatable for domesticated animals, and so will be decomposed more readily to CO_2 and make a lower contribution to soil carbon than more recalcitrant tissues. Among grass species it is the root litter that is more recalcitrant and offers a greater obstacle to decomposer organisms (Craine *et al.*, 2005). The impact upon root litter quality of selective breeding for increased WSC content of shoot tissues is as yet unknown but whether or not there is a change in decomposability is likely to affect the positive or negative outcome this trait might have on SOM. Apart from root and shoot tissues, the other factor that can influence the sequestration potential of plants is root exudation, both the quantity and quality of the compounds that are exuded. It has been suggested that root exudation is governed by plant metabolic activity (Bardgett *et al.*, 2005), with faster growing species producing more exudates. Again, the effect on root exudation of selection for a higher WSC content is little understood. Higher WSC in shoots may mean a higher concentration of WSC across all plant organs and in exudates. Alternatively, it could mean that the plant is more successful in partitioning WSC and concentrating them in shoot tissues and hence reducing concentration in roots and exudates.

Soils contain about twice as much C as the atmosphere and many soils are potentially able to sequester more than they do currently (Smith and Fang, 2010). Increasing steady-state soil C by 15% (e.g. from 0.05g/g to 0.058 g/g), it is claimed (Kell, 2011) would lower atmospheric CO_2 by 30%, leading to a large environmental benefit. Deep soil carbon is an important contributor to overall soil carbon stocks with grasses and trees acting as major sources (Chabbi *et al*., 2009; Harper and Tibbett, 2013). Gregory *et al.* (2011) estimated that 980 Mt of organic C is stored below 30cm depth in soils in England and Wales, approximately 50% of the total. Evidence from tropical savannah suggests that the planting of exotic deeper rooting plant varieties leads to significant increases in soil carbon (Fisher *et a*l., 1994). It is

helpful that a grass trait that has received recent attention by crop geneticists for a range of reasons is for deeper rooting, with focus particularly on *Festulolium* (Kell, 2011). These are hybrids or hybrid derivatives between any *Festuca* (fescue) and *Lolium* (ryegrass) species, designed for their combined complementary characters (Ghesquière *et al*., 2010). The potential of certain *Festulolium* hybrid combinations root growth and turn over to enhance SOC in sub-soils is currently being investigated and it may extend the range and diversity of soil biota. The deep rooting trait was originally selected as an aid to an improved drought resistance (Durand *et al*., 2007; Alm *et al*., 2011), but also provides other ecosystem services, such as improving soil water retention and hence reduced runoff (Gregory *et a*l., 2010; Macleod *et al.*, 2013). The cultivation of *Festulolium* with large and deep-rooting systems as an aid to increase the input of atmospheric CO_2 into agricultural soils is being assessed currently (Dungait, pers. comm.). However, there is conflicting opinion on whether the stores of SOC in subsoil will be increased or decreased by the introduction of new C. In addition, in order for deep-rooting plants to have any significant effect, the soil must be deeper than the root depth of current varieties, and furthermore root growth must not be impeded due to compaction, nutrient limitation or waterlogging. On thin soils or where roots cannot penetrate, deeper rooting plants are unlikely to provide additional sequestration benefits. Also some studies have pointed to the effect of "priming" whereby fresh labile organic matter stimulates the decomposition of older soil organic matter through the soil profile (Kuzyakov, 2010) and more specifically at depth (Fontaine et al., 2007). What happens to soil C at depth during disturbances such as drying or ploughing, land use change etc. is still little understood (Gregory *et al*., 2011).
From an agricultural perspective, it is essential that a balance between above- and below ground growth is achieved so that forage yields are not significantly compromised. Recent studies at IBERS have demonstrated that *Festulolium* hybrids involving combinations of *L. multiflorum* or *L. perenne* with either *F. arundinacea var glaucescens* or *F. mairei* have excellent agronomic performance including high yields of forage together with high WSC, and DOMD. From the perspective of their C sequestration potential, they also produce deep root systems that match their high above-ground growth. The *L. perenne* x *F. pratensis* amphiploid variety Prior has deep rooting and significant root turn-over at depth (MacLeod *et al.*, 2013) leading to potential ecosystem benefits in terms of improved soil porosity. Dungait (pers. comm.) has provided preliminary data supporting greater root depth and C deposition by *Festulolium* cv Prior in soils compared with perennial ryegrass.

Impacts of climate on C sequestration: There have been extensive reviews (e.g. Soussana and Lüscher, 2007) of the impact of multiple components of climate change, singly or in combination, on the fluxes of C and N. As the major resource for photosynthesis, the expectation borne out by field experimentation is that elevated CO_2 will increase all plant derived C fluxes into the system. An increased uptake and availability of C due to elevated CO_2 has been shown to increase C:N ratios in tissues within pasture species (Poorter *et al*., 1997) and to increase the exudation from roots of labile C (Allard *et al*., 2006). However, over an 11 year period of CO_2 enrichment of grazed pastures, Newton *et al*. (2010) found a gradual transfer of N from plant to soil pools which would undoubtedly be a constraint on plant growth responses to elevated CO_2. Overall, models predict higher total soil C sequestration in grasslands at elevated CO_2 (Pepper *et al*., 2005).
A recent study on Oklahoma prairie grasslands has shown that complex interactions occur in microbial diversity in soils in reaction to warming and drought. Warming without drought had the effects of increasing the abundance of microbes, but had the effect of reducing their diversity. Warming with drought resulted in large reductions of microbial populations but diversity was not affected. Some microbial communities are very resilient and recover

quickly after the stress is removed, but others are not, becoming dormant and requiring significant time to reinstate after the stress had been removed (Sheik *et al*., 2011).
Soil microbial composition is affected directly by the accompanying plant species in grasslands. Grigulis, *et al*. (2013) showed that high biomass production by plants was accompanied by bacterial dominance in the soil, fast microbial processes and a competitive strategy. By contrast, plant traits exhibiting low biomass production were associated with an increase in fungal communities, and slow microbial turnover. The low biomass communities were therefore conservative by nature, providing ecosystem services such as high nutrient retention but lower C sequestration, while the more exploitative plant communities provided greater Net Primary Productivity and more C sequestration.

Redesigning grassland crops for improved adaptations to climate change and for ecosystem service: Comparative studies of genetic and phenotypic diversity found in natural grasslands adapted to contrasting climates provides us with insights into the key mechanisms involved in resistance to specific abiotic stress extremes, and reveals components of their genetic control. By selection and transfer from adapted ecotypes of trait-specific alleles, centuries of evolved adaptations may frequently be utilized over a few generations to reproduce a comparable phenotype in a commercial cultivar. For a perennial grassland species, not only is cultivar performance and stability over years important, but also its ability to coexist within complementary species' mixtures and its competitive capabilities against invasive species. An extensive literature has explored the basis of grass/clover competition, with both above and below-ground factors found to be important. Shading of clover by grass can occur, depending on clover leaf size, management and, crucially, soil nitrogen (N) status. There is thus a strong interaction between interspecific plant competition and soil, particularly for the clover/ *Rhizobium* symbiosis. Hydraulic lift is another positive species interaction that could be incorporated in species mixtures. The phenomenon, reported initially amongst tree and shrub species, describes how shallow-rooting species can benefit from improved acquisition of water and nutrients because of the release of water and nutrients into the topsoil from deep-rooting neighbours (e.g. Snyder *et al.*, 2008).

Advances in plant breeding strategy at IBERS over recent years have provided productive grass and clover varieties that also safeguard the environment. In the forefront are the high sugar ryegrasses (HSG) described earlier, and also legumes designed to improve ruminant nutrition by more efficient protein conversion and to thereby reduce greenhouse gas emissions by livestock. Two possible strategies of increasing efficiency of conversion of forage N to microbial N have been used: (i) increasing the amount of readily available energy accessible during the early part of the fermentation; and (ii) providing a level of protection to the forage proteins, and thereby reducing the rate at which their breakdown products are made available to the colonizing microbial population. The HSG are examples of the former where increased WSC has been shown to have a positive impact on meat yields (Lee *et al.*, 2001) and milk production (Miller *et al.*, 2001). A significant contributor to greenhouse gas emissions by livestock is the plant-mediated proteolysis that occurs as a consequence of the assorted stresses encountered by living cells of ingested forage whilst in the rumen (Kingston-Smith *et al.*, 2010). The second strategy to improve ruminant nutrition has focused on this with a role for *Festulolium* in protein protection having been identified (O'Donovan *et al*., 2013). Amphiploid hybrids involving either *L. multiflorum* or *L. perenne* together with *Festuca arundinacea var glaucescens* have highly significantly enhanced protein stability compared with their *Lolium* parents when subjected to rumen-simulated conditions. It is hypothesised that protective measures that have evolved in the fescue to combat heat stress in

its natural Mediterranean habitat, also come into play in the rumen to provide protein protection and to provide greater time for breakdown by rumen-based microbial populations. Plant breeding initiatives to improve protein stability under rumen conditions and to reduce greenhouse gas emissions by livestock have also involved legumes, such as polyphenol oxidase (PPO) expression by red clover (*Trifolium rubra*) (Webb *et al.* 2013). Condensed tannins also help to stabilize protein as it passes through the rumen, reducing loss of protein and preventing bloat. Although absent from the leaves of white and red clover, they are present in *Lotus corniculatus* and *Lotus uliginosus* (Marshall *et al*., 2008). Extensive variation in condensed tannin content of the leaves of *Lotus* has been found. The development of varieties with appropriate levels of condensed tannins, to reduce protein loss without reducing intake or the agronomic yield and persistence is a research objective.

Developing grasses with increased resilience to climate change: Reference has already been made of the potential of *Festulolium* for ecosystem service. There are several examples of natural *Festulolium* hybrids. For example, both Italian ryegrass and perennial ryegrass hybrids with meadow fescue occur naturally in the UK as *Festulolium braunii* and *Festulolium loliaceum*, respectively and have evolved with certain adaptive capabilities superior to their parent species. *F. loliaceum* for example, is found commonly in mature meadows in flood-prone highly water-logged soils (Humphreys and Harper, 2008). IBERS achieved a notable first in 2012 in gaining inclusion of the *Festulolium* variety AberNiche into the UK National Recommended Varieties List which is a modified *F. braunii* comprising circa 10% meadow fescue genome (based on genetic and cytological screens fescue-specific using DarT markers and genome in-situ hybridisation (GISH) (Kopecky and Harper, unpublished). The variety was developed for its high forage production and improved winter hardiness, presumably derived from its cold-tolerant fescue progenitor, but has also demonstrated excellent resilience to heat and drought.
The entry of variety AberNiche onto the UK National Recommended Varieties List heralds a new dawn for *Festulolium* breeding in the UK. Genes for drought resistance have been transferred successfully from both *F. arundinacea var glaucescens* and from *F. arundinacea* onto chromosome 3 of both *L. multiflorum* and *L. perenne* and have improved the water use efficiency of both ryegrasses by >80% (Humphreys *et al.* 2013). Breeders' lines with these fescue gene complements are currently in trial for variety assessment.

Impact of flooding and drought: The use of *Festulolium* for flood mitigation has been recently proposed (Macleod *et. al.*, 2013). In the UK, the cost of flooding is significant; it has been estimated that the devastating floods of summer 2007 cost the UK economy £3.2b (Environment Agency). Excessive run-off erodes top-soils and soil organic matter, and depletes valuable nutrients, with negative impacts on water quality. Eutrophication of surface and ground-waters in England and Wales is estimated to cost £75-114m a^{-1} due to loss of amenity value, reduced biodiversity and increased costs of water treatment. The cost of damage to agricultural soil in England and Wales has been estimated as £264m a^{-1}, and that of treating water contaminated with agricultural pollutants as £203m a^{-1} (UK Parliamentary Office of Science & Technology, 2006). The winter of 2013-2014 brought record rainfall to the UK leading to extreme flooding events across largely grassland-dominated areas. Insurance losses for England and Wales are expected to amount to £1.2b. Over the UK as a whole £600m of crops were lost due to flooding in 2012. To help mitigate the worst effects of flooding, *Festulolium* hybrid combinations are proving effective. Macleod *et al.* (2013) demonstrated how *L. perenne* x *F. pratensi*s variety Prior reduced rainfall surface run-off by 51% compared to an IBERS-bred elite perennial ryegrass variety. It is hypothesised that the root turn-over at depth of the *Festulolium* led to improved soil structure and porosity and to

improved soil water retention and reduced overland run-off. In a new five year project (2014-2018) called SUREROOT, funded jointly by BBSRC and industry, IBERS and Rothamsted Research are assessing the potential of *Festulolium* and clover varieties both independently and in combination for flood mitigation. The trial will include field assessments on farms at different UK locations, on contrasting soils, and with alternative livestock management practice. The project also employs use of IBERS new National Plant Phenomics Centre and the North Wyke Farm Platform to assess how modified root systems on individual plant genotypes may if reproduced at the field scale affect soil structure and water, nutrient, and C run-off at the field scale. Similar to *Festulolium* cv Prior, white clover improves soil structure and drainage through improved soil aggregation, which increases soil porosity and water infiltration (Holtham *et al.* 2007; Mytton *et al.* 1993). Values of macroporosity for soil cores under perennial ryegrass and under white clover were reported as 24% and 45%, respectively, indicating a significant improvement in water drainage through use of white clover (Holtham *et al*. 2007).

Festulolium for bioremediation on mine-spoil: Whilst primarily for economic reasons, mining underground for coal in the UK is largely no longer practised, it has been replaced by open-cast mining in areas such as South Wales, a practice viewed as a controversial due to its unsightly look, the large land areas employed, and the potential damage to both landscape and environment. It is considered a priority in such locations, that areas used for open cast mining are restored to their natural grassland ecology as quickly as possible as soon as coal extraction has ceased. However, combinations of a challenging climate and growth inhibiting mine-spoils have restricted progress to land reclamation. However new *Festulolium* combinations: *L. perenne* x *F. pratensis, L. perenne* x *F. arundinacea var glaucescens,* and *L. perenne* x *F. mairei* amphiploid hybrids are being assessed by IBERS in trials on over-burden mounds on open-cast mine workings in South Wales aimed at their bioremediation and their restoration to grasslands. The combination of stress tolerance, rapid establishment and growth, nutrient and water-use-efficiency, and large strong root systems present in the *Festulolium* hybrids provide them with an advantage over native grasses. Their rapid root turn-over should provide new sources of C to the mine spoil and provide the foundation for indigenous UK grasses to colonise and eventually return the land finally to its natural condition.

Conclusion: The development of new grassland agro-ecosystems must take account not only of traditional values of production, disease resistance, persistence and forage quality but also their impact on their surrounding environment. National variety assessment needs to be amended to account for the potential benefits to accrue through the use of novel varieties that offer enhanced resilience against climate change and/or provisions for other ecosystem service. A cohesive strategy is required involving relevant stakeholders to inform, encourage, and reward farmers to grow grassland varieties that provide a specific ecosystem service such as flood mitigation, to provide the necessary guidance as to where to sow, and how to manage sufficiently to produce the optimal benefits.
Anthropomorphic and natural grasslands can potentially be mutually advantageous. Whilst historically agricultural developments have diminished areas of natural grassland and have led to the loss of their traditional environmental provisions, a reverse result may also be achieved. Novel grassland varieties have potential to engineer conditions that allow the restoration of natural grassland ecosystems e.g. in land reclamation from industrially contaminated “brown-field sites”. They may also extend the depth of sub-soils and extend the range of soil biota by rooting deeper than indigenous species. Indeed they may in species mixtures increase the resilience of grassland ecosystems to climate change in situations where the indigenous species lack the necessary adaptations to the stresses likely to be encountered.

The genetic resources of many 'semi-natural' grassland species can be harnessed to bring both the productive and 'unproductive' grasslands more into line. Semi-natural grasslands should not be showcased and "fossilised in time and place" but must be preserved as essential resources for genetic material for future crop improvement and for ameliorating the damage done by traditional productive systems. Restoration at the landscape scale is required to redress the balance and provide more connectivity, more ecosystem services, and more multifunctional dynamism in grassland management.

Whilst new grassland varieties are required to reduce our 'agricultural footprint', to safeguard the future of fragile rural communities and to sustain our livestock industry, it is essential that further safeguards are put in place to assist the perpetuity of our existing natural and semi-natural grasslands. In the current drive to increase productivity, natural grasslands are in danger of being further eroded and fragmented, greatly reducing their biodiversity and their ecosystem service value. They it must be remembered provide a much more diverse range of essential ecosystem services than those available within agricultural crop based systems. Indeed, restoration of partially-degraded grasslands should also form part of national objectives. Grasslands, both natural and cultivated, dominate our landscape and both have a vital role in safeguarding the environment on which we all depend.

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